09/700712

(FILE 'CAPLUS' ENTERED AT 14:23:49 ON 30 JUL 2004) - key terms
L1 385 S THYA OR (THY OR THYMINE)(W)A
L2 9 S L1 AND CHOLERAE

L2 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 05 Oct 2003
ACCESSION NUMBER: 2003:778638 CAPLUS
DOCUMENT NUMBER: 139:349472
TITLE: Construction and evaluation of a safe, live, oral Vibrio **cholerae** vaccine candidate, IEM108
AUTHOR(S): Liang, Weili; Wang, Shixia; Yu, Fenggang; Zhang, Lijuan; Qi, Guoming; Liu, Yanqing; Gao, Shouyi; Kan, Biao
CORPORATE SOURCE: Priority Laboratory of Medical Molecular Bacteriology of the Ministry of Health, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, 102206, Peop. Rep. China
SOURCE: Infection and Immunity (2003), 71(10), 5498-5504
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB IEM101, a Vibrio **cholerae** O1 El Tor Ogawa strain naturally deficient in CTXΦ, was previously selected as a live cholera vaccine candidate. To make a better and safer vaccine that can induce protective immunity against both the bacteria and cholera toxin (CT), a new vaccine candidate, IEM108, was constructed by introducing a ctxB gene and an El Tor-derived rstR gene into IEM101. The ctxB gene codes for the protective antigen CTB subunit, and the rstR gene mediates phage immunity. The stable expression of the two genes was managed by a chromosome-plasmid lethal balanced system based on the housekeeping gene **thyA**. Immunization studies indicate that IEM108 generates good immune responses against both the bacteria and CT. After a single-dose intraintestinal vaccination with 109 CFU of IEM108, both anti-CTB IgG and vibriocidal antibodies were detected in the immunized-rabbit sera. However, only vibriocidal antibodies are detected in rabbits immunized with IEM101. In addition, IEM108 but not IEM101 conferred full protection against the challenges of four wild-type toxigenic strains of V. **cholerae** O1 and 4 μg of CT protein in a rabbit model. By introducing the rstR gene, the frequency of conjugative transfer of a recombinant El Tor-derived RS2 suicidal plasmid to IEM108 was decreased 100-fold compared to that for IEM101. This indicated that the El Tor-derived rstR cloned in IEM108 was fully functional and could effectively inhibit the El Tor-derived CTXΦ from infecting IEM108. The authors' results demonstrate that IEM108 is an efficient and safe live oral cholera vaccine candidate that induces antibacterial and antitoxic immunity and CTXΦ phage immunity.
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

Searcher : Shears 571-272-2528

L2 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 27 Jan 2003
ACCESSION NUMBER: 2003:62239 CAPLUS
DOCUMENT NUMBER: 139:83574
TITLE: Construction and characterisation of O139 cholera vaccine candidates
AUTHOR(S): Ledon, Talena; Valle, Edgar; Valmaseda, Tania; Cedre, Barbara; Campos, Javier; Rodriguez, Boris L.; Marrero, Karen; Garcia, Hilda; Garcia, Luis; Fando, Rafael
CORPORATE SOURCE: Grupo de Genetica, Centro Nacional de Investigaciones Cientificas, Havana, 6412, Cuba
SOURCE: Vaccine (2003), 21(11-12), 1282-1291
CODEN: VACCDE; ISSN: 0264-410X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The hemagglutinin/protease (HA/P) seems to be an attractive locus for the insertion of heterologous tags in live cholera vaccine strains. A ΔCTXΦ spontaneous mutant derived from a pathogenic strain of O139 Vibrio **cholerae** was sequentially manipulated to obtain hapA:celA derivs. which were later improved in their environmental safety by a **thyA** mutation. All the strains here obtained showed similar phenotypes in traits known to be remarkable for live cholera vaccines irresp. of their motility phenotypes, although the hapA mutants had a 10-fold decrease in their colonization capacity compared with their parental strains in the infant mouse cholera model. However, the subsequent **thyA** mutation did not affect their colonization properties in the same model. These preliminary results pave the way for further clin. assays to confirm the possibilities of these vaccine prototypes as safe and effective tools for the prevention of O139 cholera.
REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 06 Nov 2000
ACCESSION NUMBER: 2000:776456 CAPLUS
DOCUMENT NUMBER: 134:39257
TITLE: Construction and characterization of a nonproliferative El Tor cholera vaccine candidate derived from strain 638
AUTHOR(S): Valle, Edgar; Ledon, Talena; Cedre, Barbara; Campos, Javier; Valmaseda, Tania; Rodriguez, Boris; Garcia, Luis; Marrero, Karen; Benitez, Jorge; Rodriguez, Sandra; Fando, Rafael
CORPORATE SOURCE: Grupo de Genetica, Centro Nacional de Investigaciones Cientificas, Havana, Cuba
SOURCE: Infection and Immunity (2000), 68(11), 6411-6418
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In recent clin. assays, our cholera vaccine candidate strain, Vibrio

cholerae 638 El Tor Ogawa, was well tolerated and immunogenic in Cuban volunteers. In this work we describe the construction of 638T, a thymidine auxotrophic version of improved environmental biosafety. In so doing, the **thyA** gene from V. **cholerae** was cloned, sequenced, mutated in vitro, and used to replace the wild-type allele. Except for its dependence on thymidine for growth in minimal medium, 638T is essentially indistinguishable from 638 in the rate of growth and morphol. in complete medium. The two strains showed equivalent phenotypes with regard to motility, expression of the celA marker, colonization capacity in the infant mouse cholera model, and immunogenicity in the adult rabbit cholera model. However, the ability of this new strain to survive environmental starvation was limited with respect to that of 638. Taken together, these results suggest that this live, attenuated, but nonproliferative strain is a new, promising cholera vaccine candidate.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 24 Aug 2000
ACCESSION NUMBER: 2000:586120 CAPLUS
DOCUMENT NUMBER: 134:203211
TITLE: Development of a chromosome-plasmid balanced lethal gene expression system of Vibrio **cholerae** based on **thyA** locus
AUTHOR(S): Xia, Xiaobin; Qi, Guoming; Liu, Yanqing; Gao, Shouyi
CORPORATE SOURCE: Institute of Epidemiology and Microbiology, Chinese academy of Preventive Medicine, Beijing, 102206, Peop. Rep. China
SOURCE: Zhonghua Weishengwuxue He Mianyixue Zazhi (2000), 20(3), 223-227
CODEN: ZWMZDP; ISSN: 0254-5101
PUBLISHER: Weishenbu Beijing Shengwu Zhipin Yanjiuso
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB Objective: To make up a delivery vector for construction of live oral Vibrio **cholerae** vaccine candidate. Methods: In a modified MM minimal medium, a Vibrio **cholerae** strain IEM101 of **thyA** gene mutant (PL102) was screened by adding trimethoprim (TMP) 15μg/mL and thymidine 50μg/mL. The PCR product of the **thyA** gene of E. coli was cloned into pUC18(pXXB106) and then transformed into PL102 by electroporation. Results: The chromosome-plasmid balanced lethal gene expression system of Vibrio **cholerae** based on **thyA** locus was constructed. Conclusion: The balanced system was defined as a delivery vector candidate for further study.

L2 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 03 Dec 1999
ACCESSION NUMBER: 1999:764211 CAPLUS
DOCUMENT NUMBER: 132:19629
TITLE: Production of **ThyA**- strains of Vibrio **cholerae** and their use for expression of

heterologous proteins
INVENTOR(S): Carlin, Nils; Lebens, Michael R.
PATENT ASSIGNEE(S): SBL Vaccin AB, Swed.
SOURCE: PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9961634	A1	19991202	WO 1999-EP3509	19990521
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2329255	AA	19991202	CA 1999-2329255	19990521
AU 9944999	A1	19991213	AU 1999-44999	19990521
BR 9910703	A	20010130	BR 1999-10703	19990521
EP 1080211	A1	20010307	EP 1999-927745	19990521
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, LT, LV, FI				
EE 200000686	A	20020415	EE 2000-686	19990521
NO 2000005950	A	20010125	NO 2000-5950	20001124

PRIORITY APPLN. INFO.: SE 1998-1852 A 19980526
WO 1999-EP3509 W 19990521

AB A method of producing a **thyA**-deficient (**thyA**-) strain of Vibrio **cholerae** comprising site-directed mutagenesis in the V. **cholerae** chromosome at the locus of the **thyA** gene. Particularly, a .DELTA.**thyA** strain of Vibrio **cholerae** lacking the thymidylate synthetase functionality of the **thyA** is disclosed. Knowledge of the **thyA** and surrounding sequences allows the use of suitable suicide vectors for site-directed mutagenesis and strategies such as (1) insertional inactivation, (b) a combination of insertional inactivation and gene deletion, and (c) removal of the entire gene. The **thyA**- strain may comprise one or several episomal autonomously replicating DNA elements, such as plasmids, having an optionally foreign, e.g. Escherichia coli, functional **thyA** gene that enables the strain to grow in the absence of thymine in the growth medium, and optionally having a structural gene encoding a homologous or heterologous protein. Further, proteins encoded by a structural **thyA** gene and the 5'-flanking region are described. Addnl., a vaccine comprising a Vibrio **cholerae** .DELTA.**thyA** strain of the invention or a **thyA**- strain of Vibrio **cholerae** produced by the method of the invention is disclosed. Thus, a useful application of the **thyA** gene is in maintenance of recombinant plasmids employed in the overprodn. of recombinant proteins in V. **cholerae**, and in the use of the sequence

for insertion of foreign genes in a selectable and site-specific manner into the V. **cholerae** chromosome. This system is exemplified by insertion and expression of the heat-labile enterotoxin B-subunit of Escherichia coli and the glutathione S-transferase 26-kDa protein of Schistosoma japonica.

L2 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 23 Jul 1999
ACCESSION NUMBER: 1999:451377 CAPLUS
DOCUMENT NUMBER: 131:98485
TITLE: Novel strain of Vibrio **cholerae** and its use in humans as a live oral vaccine against cholera
INVENTOR(S): Campos Gomez, Javier; Fando Calzada, Rafael Alfredo; Rodriguez Gonzalez, Boris Luis; Ledon Perez, Talena Yamile; Valle Diaz, Edgar; Silva Cabrera, Anisia Juana; Benitez Robles, Jorge Antonio
PATENT ASSIGNEE(S): Centro Nacional de Investigaciones Cientificas, Cuba
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Spanish
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9935271	A2	19990715	WO 1998-CU8	19981230
WO 9935271	A3	19991125		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9919591	A1	19990726	AU 1999-19591	19981230
EP 1099759	A2	20010516	EP 1998-964347	19981230
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002500047	T2	20020108	JP 2000-527655	19981230
BR 9814600	A	20020219	BR 1998-14600	19981230
US 6723323	B1	20040420	US 2000-582772	20001204

PRIORITY APPLN. INFO.: CU 1997-142 A 19971230
WO 1998-CU8 W 19981230

AB The present invention describes a method of obtaining a strain of Vibrio **cholerae** that can be used as a live oral vaccine to inoculate humans against cholera. Said strain is a mutant of Vibrio cholera wherein the hemagglutinin protease gene (hap) is inactivated by the insertion of a marker gene (ce/A) into the coding region. The invention also includes methods of minimizing the impact of the genetically engineered strain on the environment. For such

purposes, a double mutant is made by creating an internal deletion within the gene **thyA**, which thereby suppresses the expression of thymidylate synthase.

L2 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 08 Dec 1995
ACCESSION NUMBER: 1995:970438 CAPLUS
DOCUMENT NUMBER: 124:4745
TITLE: Thymine auxotrophy as an attenuating marker in Vibrio **cholerae**
AUTHOR(S): Attridge, S. R.
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Adelaide, Adelaide, 5005, Australia
SOURCE: Microbial Pathogenesis (1995), 19(1), 11-18
CODEN: MIPAEV; ISSN: 0882-4010
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Vibrio **cholerae** CVD102 is a thymine-dependent auxotroph of CVD101, a cholera toxin A-B+ candidate live oral cholera vaccine. Previous clin. experience with these strains suggested that, by restricting intestinal growth, thymine auxotrophy is attenuating for V. **cholerae**. Studies in the infant mouse cholera model cast doubt upon this conclusion however. Stable **thyA** mutants selected from each of three pathogenic strains showed unimpaired gut colonization in mixed-infection competition expts. Similar results were obtained using **thyA** mutants selected from two atoxigenic strains, including CVD101. Further studies with CVD102 showed that the reduced colonization potential of this strain could not be compensated by the provision of a functional **thyA**+ gene in trans. CVD102 shows reduced synthesis of toxin-coregulated pili (TCP) during in vitro growth, suggesting the presence of a second, undefined mutation in this strain. Given the critical role of TCP in intestinal colonization, it seems probable that this previously unrecognized mutation is responsible for the poor in vivo performance of CVD102.

L2 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 21 Feb 1992
ACCESSION NUMBER: 1992:56898 CAPLUS
DOCUMENT NUMBER: 116:56898
TITLE: Construction of plasmid vectors with a non-antibiotic selection system based on the Escherichia coli **thyA**+ gene: application to cholera vaccine development
AUTHOR(S): Morona, Renato; Yeadon, Jane; Considine, Andrew; Morona, Judy K.; Manning, Paul A.
CORPORATE SOURCE: Enterovax Ltd., Adelaide, 5001, Australia
SOURCE: Gene (1991), 107(1), 139-44
CODEN: GENED6; ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The construction of live oral carriers based on attenuated Salmonella strains as vectors offers a new approach to vaccine development. A set of plasmid vectors was constructed which have

the **thyA** gene of E. coli (encoding thymidylate synthetase) as the marker for selection and maintenance of plasmid clones. The **thyA** system offers an alternative to antibiotic-resistance selection markers. It can be easily adapted to a particular host-vector combination since **thyA** chromosomal mutations can be readily introduced by trimethoprim selection. **thyA** -Based plasmids were constructed with the Vibrio **cholerae** rfb genes (encoding O-antigen biosynthesis of the Inaba serotype). These have been found to be useful in the construction of candidate bivalent cholera-typhoid vaccines.

L2 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
ED Entered STN: 24 Jun 1988
ACCESSION NUMBER: 1988:217250 CAPLUS
DOCUMENT NUMBER: 108:217250
TITLE: Construction of plasmid vectors containing non-antibiotic selection markers and their use for expression of antigen genes
INVENTOR(S): Morona, Renato; Manning, Paul A.
PATENT ASSIGNEE(S): Enterovax Research Pty. Ltd., Australia
SOURCE: Eur. Pat. Appl., 14 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 251579	A2	19880107	EP 1987-305404	19870618
EP 251579	A3	19890322		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AU 8774202	A1	19880107	AU 1987-74202	19860624
AU 594161	B2	19900301		
DK 8703188	A	19871225	DK 1987-3188	19870623
JP 63039588	A2	19880220	JP 1987-157463	19870624

PRIORITY APPLN. INFO.: AU 1986-6553 19860624

AB Plasmid cloning vectors containing a nonantibiotic marker gene consisting of a nonreverting **thyA**+ gene are constructed. Plasmid pEVX1 was constructed by inserting the ompV gene of Vibrio **cholerae** from plasmid pOmpV210 in the ScaI site, and the tyA+ gene from plasmid pBTAH in the HindIII site of pBR322. The tetracycline resistance gene was inactivated by digestion with NaeI followed by religation. Salmonella typhimurium EX143 was transformed with the plasmid and selected for **ThyA**+. The resulting transformants expressed high levels of OmpV protein.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXCENTER, PASCAL, DISSABS, FEDRIP' ENTERED AT 14:25:51 ON 30 JUL 2004)
L3 33 S L2
L4 11 DUP REM L3 (22 DUPLICATES REMOVED)

L4 ANSWER 1 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2004:257862 BIOSIS

DOCUMENT NUMBER: PREV200400258028
TITLE: Vibrio **Cholerae** vaccine candidates and method of their constructing.
AUTHOR(S): Gomez, Javier Campos [Inventor, Reprint Author]; Calzada, Rafael Alfredo Fando [Inventor]; Gonzalez, Boris Luis Rodriguez [Inventor]; Diaz, Edgar Valle [Inventor]; Perez, Talena Yamile Ledon [Inventor]; Silva, Anisia Juana [Inventor]; Robles, Jorge Antonio Benitez [Inventor]
CORPORATE SOURCE: Villa Clara, Cuba
ASSIGNEE: Centro Nacional de Investigaciones Cientificas, (CNIC), Cuba
PATENT INFORMATION: US 6723323 April 20, 2004
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Apr 20 2004) Vol. 1281, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file.
ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 12 May 2004
Last Updated on STN: 12 May 2004

AB Vibrio **cholerae** vaccine strains which have a disrupted hap gene and which are tagged with celA coding functions from Clostridium thermocellum are described. A contained, genetically defined **thyA** mutant of Vibrio **cholerae** and the general methodology of making along with the sequence of **thyA** gene are also described.

L4 ANSWER 2 OF 11 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003440542 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14500467
TITLE: Construction and evaluation of a safe, live, oral Vibrio **cholerae** vaccine candidate, IEM108.
AUTHOR: Liang Weili; Wang Shixia; Yu Fenggang; Zhang Lijuan; Qi Guoming; Liu Yanqing; Gao Shouyi; Kan Biao
CORPORATE SOURCE: Priority Laboratory of Medical Molecular Bacteriology, Ministry of Health, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, People's Republic of China.
SOURCE: Infection and immunity, (2003 Oct) 71 (10) 5498-504. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200311
ENTRY DATE: Entered STN: 20030923
Last Updated on STN: 20031104
Entered Medline: 20031103

AB IEM101, a Vibrio **cholerae** O1 El Tor Ogawa strain naturally deficient in CTXPhi, was previously selected as a live cholera vaccine candidate. To make a better and safer vaccine that can induce protective immunity against both the bacteria and cholera toxin (CT), a new vaccine candidate, IEM108, was constructed by

introducing a ctxB gene and an El Tor-derived rstR gene into IEM101. The ctxB gene codes for the protective antigen CTB subunit, and the rstR gene mediates phage immunity. The stable expression of the two genes was managed by a chromosome-plasmid lethal balanced system based on the housekeeping gene **thyA**. Immunization studies indicate that IEM108 generates good immune responses against both the bacteria and CT. After a single-dose intraintestinal vaccination with 10(9) CFU of IEM108, both anti-CTB immunoglobulin G and vibriocidal antibodies were detected in the immunized-rabbit sera. However, only vibriocidal antibodies are detected in rabbits immunized with IEM101. In addition, IEM108 but not IEM101 conferred full protection against the challenges of four wild-type toxigenic strains of V. **cholerae** O1 and 4 micro g of CT protein in a rabbit model. By introducing the rstR gene, the frequency of conjugative transfer of a recombinant El Tor-derived RS2 suicidal plasmid to IEM108 was decreased 100-fold compared to that for IEM101. This indicated that the El Tor-derived rstR cloned in IEM108 was fully functional and could effectively inhibit the El Tor-derived CTXPhi from infecting IEM108. Our results demonstrate that IEM108 is an efficient and safe live oral cholera vaccine candidate that induces antibacterial and antitoxic immunity and CTXPhi phage immunity.

L4 ANSWER 3 OF 11 MEDLINE on STN DUPLICATE 2

ACCESSION NUMBER: 2003095708 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12559810
TITLE: Construction and characterisation of O139 cholera vaccine candidates.
AUTHOR: Ledon Talena; Valle Edgar; Valmaseda Tania; Cedre Barbara; Campos Javier; Rodriguez Boris L; Marrero Karen; Garcia Hilda; Garcia Luis; Fando Rafael
CORPORATE SOURCE: Grupo de Genetica, Centro Nacional de Investigaciones Cientificas, AP 6412 Havana, Cuba.. talena@biocnic.cneuro.edu.cu
SOURCE: Vaccine, (2003 Mar 7) 21 (11-12) 1282-91. Journal code: 8406899. ISSN: 0264-410X.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200310
ENTRY DATE: Entered STN: 20030302
Last Updated on STN: 20031101
Entered Medline: 20031031

AB The hemagglutinin/protease (HA/P) seems to be an attractive locus for the insertion of heterologous tags in live cholera vaccine strains. A deltaCTXphi spontaneous mutant derived from a pathogenic strain of O139 Vibrio **cholerae** was sequentially manipulated to obtain hapA Colon, two colons celA derivatives which were later improved in their environmental safety by means of a **thyA** mutation. All the strains here obtained showed similar phenotypes in traits known to be remarkable for live cholera vaccines irrespective of their motility phenotypes, although the hapA mutants had a 10-fold decrease in their colonisation capacity compared with their parental strains in the infant mouse cholera model. However, the subsequent **thyA** mutation did not

affect their colonisation properties in the same model. These preliminary results pave the way for further clinical assays to confirm the possibilities of these vaccine prototypes as safe and effective tools for the prevention of O139 cholera.

L4 ANSWER 4 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 3

ACCESSION NUMBER: 2003:511581 BIOSIS
DOCUMENT NUMBER: PREV200300514621
TITLE: Construction and evaluation of the biosafe and live oral vaccine candidate of El Tor Vibrio **cholerae** IEM108.
AUTHOR(S): Liang Wei-li; Kan Biao [Reprint Author]; Yu Feng-gang; Qi Guo-Ming; Liu Yan-qing; Gao Shou-Yi
CORPORATE SOURCE: Priority Laboratory of Medical Molecular Bacteriology, Ministry of Health, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, 102206, China
kanb@btamail.net.cn
SOURCE: Zhonghua Weishengwuxue He Mianyixue Zazhi, (July 2003) Vol. 23, No. 7, pp. 522-528. print.
CODEN: ZWMZDP. ISSN: 0254-5101.
DOCUMENT TYPE: Article
LANGUAGE: Chinese
ENTRY DATE: Entered STN: 5 Nov 2003
Last Updated on STN: 5 Nov 2003

AB Objective: To develop an improved attenuated oral Vibrio **cholerae** vaccine candidate which is immune to CTXPHI infection and elicits both antibacterial and antitoxic immunity. Methods: Based on the non-toxigenic and **thyA** gene deletion strain IEM101-T developed from IEM101, an El Tor biotype vaccine candidate, we constructed a chromosome-plasmid balanced lethal system by using **thyA** gene of E. coli as selection pressure to clone rstR gene, encoding CTXPHI phage immunity, and ctxB gene, encoding cholera toxin subunit B. In immunized rabbits, anti-CTB IgG antibody and vibriocidal antibody were detected to evaluate the immunogenicity of IEM108. A control-challenged study in rabbits was used to estimate the protection of IEM108. Results: The recombinant plasmid carrying ctxB, rstR and E. coli **thyA**' was stabley maintained in IEM101-T. CTB was detected by GM1-ELISA and expressed. Animal experiments showed that IEM108 could trigger high level of the serum anti-CTB IgG antibody and vibriocidal antibody, and offered full protection against challenges with 4 wild-type toxigenic strain of different biotypes and serogroups, and at least 4mug CT. Conclusion: By using a chromosome-plasmid balanced lethal system, a biosafe and live oral Vibrio **cholerae** vaccine candidate, IEM108 was constructed, which has induced immunity to CTXPHI infection and expresses CTB subunit stabely. Animal test showed that IEM108 was safe, immunogenic and highly protective and seemed be a well-prospective candidate eliciting both antibacterial and antitoxic immunity.

L4 ANSWER 5 OF 11 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2001027227 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11035753
TITLE: Construction and characterization of a nonproliferative El Tor cholera vaccine candidate derived from strain 638.
AUTHOR: Valle E; Ledon T; Cedre B; Campos J; Valmaseda T; Rodriguez B; Garcia L; Marrero K; Benitez J; Rodriguez S; Fando R
CORPORATE SOURCE: Grupo de Genetica, Centro Nacional de Investigaciones Cientificas, Havana, Cuba.
SOURCE: Infection and immunity, (2000 Nov) 68 (11) 6411-8. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Y17135
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001115

AB In recent clinical assays, our cholera vaccine candidate strain, Vibrio **cholerae** 638 El Tor Ogawa, was well tolerated and immunogenic in Cuban volunteers. In this work we describe the construction of 638T, a thymidine auxotrophic version of improved environmental biosafety. In so doing, the **thyA** gene from V. **cholerae** was cloned, sequenced, mutated in vitro, and used to replace the wild-type allele. Except for its dependence on thymidine for growth in minimal medium, 638T is essentially indistinguishable from 638 in the rate of growth and morphology in complete medium. The two strains showed equivalent phenotypes with regard to motility, expression of the celA marker, colonization capacity in the infant mouse cholera model, and immunogenicity in the adult rabbit cholera model. However, the ability of this new strain to survive environmental starvation was limited with respect to that of 638. Taken together, these results suggest that this live, attenuated, but nonproliferative strain is a new, promising cholera vaccine candidate.

L4 ANSWER 6 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 5
ACCESSION NUMBER: 2000:314118 BIOSIS
DOCUMENT NUMBER: PREV200000314118
TITLE: Development of a chromosome-plasmid balanced lethal gene expression system of Vibrio **cholerae** based on **thyA** locus.
AUTHOR(S): Xia Xiaobin; Qi Guoming; Liu Yanqing [Reprint author]
CORPORATE SOURCE: Institute of Epidemiology and Microbiology, Chinese Academy of Preventive Medicine, Beijing, 102206, China
SOURCE: Zhonghua Weishengwuxue He Mianyixue Zazhi, (May, 2000) Vol. 20, No. 3, pp. 223-227. print.
CODEN: ZWMZDP. ISSN: 0254-5101.
DOCUMENT TYPE: Article
LANGUAGE: Chinese
ENTRY DATE: Entered STN: 26 Jul 2000
Last Updated on STN: 7 Jan 2002

Searcher : Shears 571-272-2528

AB Objective: To make up a delivery vector for construction of live oral Vibrio cholorae vaccine candidate. Methods: In a modified MM minimal medium, a Vibrio **cholerae** strain IEM101 of **thyA** gene mutant (PL102) was screened by adding trimethoprim (TMP) 15mug/ml and thymidine 50mug/ml. The PCR product of the **thyA** gene of E. coli was cloned into pUC18(pXXB106) and then transformed into PL102 by electroporation. Results: The chromosome-plasmid balanced lethal gene expression system of Vibrio **cholerae** based on **thyA** locus was constructed. Conclusion: The balanced system was defined as a delivery vector candidate for further study.

L4 ANSWER 7 OF 11 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
DUPLICATE 6
ACCESSION NUMBER: 2000-062719 [05] WPIDS
DOC. NO. CPI: C2000-017506
TITLE: New Vibrio **cholerae** strain defective in the **thyA** gene, for use in vaccines and for recombinant protein production.
DERWENT CLASS: B04 D16
INVENTOR(S): CARLIN, N; LEBENS, M R
PATENT ASSIGNEE(S): (SBLV-N) SBL VACCIN AB; (ACTI-N) ACTIVE BIOTECH AB
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9961634	A1	19991202	(200005)*	EN	42
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9944999	A	19991213	(200020)		
BR 9910703	A	20010130	(200110)		
EP 1080211	A1	20010307	(200114)	EN	
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LT LU LV MC NL PT SE					
NO 2000005950	A	20010125	(200118)		
CZ 2000004354	A3	20010516	(200132)		
HU 2001002310	A2	20011029	(200175)		
MX 2000011604	A1	20020401	(200363)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9961634	A1	WO 1999-EP3509	19990521
AU 9944999	A	AU 1999-44999	19990521
BR 9910703	A	BR 1999-10703	19990521
		WO 1999-EP3509	19990521
EP 1080211	A1	EP 1999-927745	19990521
		WO 1999-EP3509	19990521
NO 2000005950	A	WO 1999-EP3509	19990521
		NO 2000-5950	20001124
CZ 2000004354	A3	WO 1999-EP3509	19990521

		CZ 2000-4354	19990521
HU 2001002310	A2	WO 1999-EP3509	19990521
		HU 2001-2310	19990521
MX 2000011604	A1	WO 1999-EP3509	19990521
		MX 2000-11604	20001124

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9944999	A	Based on	WO 9961634
BR 9910703	A	Based on	WO 9961634
EP 1080211	A1	Based on	WO 9961634
CZ 2000004354	A3	Based on	WO 9961634
HU 2001002310	A2	Based on	WO 9961634
MX 2000011604	A1	Based on	WO 9961634

PRIORITY APPLN. INFO: SE 1998-1852 19980526

AN 2000-062719 [05] WPIDS

AB WO 9961634 A UPAB: 20000128

NOVELTY - A Vibrio **cholerae thyA**-negative strain (A) which is a Delta **thyA** strain lacking **thyA** gene functions, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) method of producing (A) by site-directed mutagenesis of the V. **cholerae** chromosome to delete and/or insert nucleotides at the **thyA** locus;

(2) the **thyA** gene (I; 2909 bp sequence given in the specification);

(3) sequence of the 5'-flanking region of (I) (838 bp sequence given in the specification);

(4) sequence of the 3'-flanking region (IV) of (I) (1222 bp sequence given in the specification);

(5) protein (Ia) encoded by (I), 283 amino acid sequence, given in the specification;

(6) protein (IIa) encoded by (II), 271 amino acid sequence, given in the specification; and

(7) a vaccine containing (A).

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Induction of a specific immune response.

USE - The Vibrio **cholerae thyA**-negative strain (A) are used:

(i) for overproduction of recombinant proteins; and

(ii) in vaccines to prevent or treat cholera (or other diseases if engineered to express the appropriate proteins).

The **thyA** gene (I) is also useful for insertion of foreign genes, in a selective and site-specific manner, and the proteins expressed by (I), or by its 5'-flanking region, are useful in research and as targets for antimicrobial therapy.

ADVANTAGE - When used for recombinant protein production, V. **cholerae** provides high yields with secretion of products into the culture medium for ease of subsequent recovery. The Vibrio **cholerae thyA**-negative strain (A) can be maintained by thymine complementation, eliminating the need for antibiotic selection.

Dwg.0/17

L4 ANSWER 8 OF 11 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 1999-430398 [36] WPIDS
DOC. NO. CPI: C1999-126864
TITLE: Producing strains of Vibrio **cholerae** with inactivated gene for hemagglutinin protease, useful in vaccines against cholera.
DERWENT CLASS: B04 D16
INVENTOR(S): CABRERA, A J S; CALZADA, R A F; DIAZ, E V; GOMEZ, J C; GONZALEZ, B L R; PEREZ, T Y L; ROBLES, J A B; BENITEZ ROBLES, J A; CAMPOS GOMEZ, J; FANDO CALZADA, R A; LEDON PEREZ, T Y; RODRIGUEZ GONZALEZ, B L; SILVA CABRERA, A J; VALLE DIAZ, E; SILVA, A J
PATENT ASSIGNEE(S): (NAIN-N) CENT NACIONAL INVESTIGACIONES CIENTIFICA
COUNTRY COUNT: 82
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9935271	A2	19990715	(199936)*	ES	30
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9919591	A	19990726	(199952)		
EP 1099759	A2	20010516	(200128)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
CN 1301301	A	20010627	(200158)		
JP 2002500047	W	20020108	(200206)		38
BR 9814600	A	20020219	(200222)		
AU 2003208135	A1	20030814	(200420)#		
US 6723323	B1	20040420	(200427)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9935271	A2	WO 1998-CU8	19981230
AU 9919591	A	AU 1999-19591	19981230
EP 1099759	A2	EP 1998-964347	19981230
		WO 1998-CU8	19981230
CN 1301301	A	CN 1998-813442	19981230
JP 2002500047	W	WO 1998-CU8	19981230
		JP 2000-527655	19981230
BR 9814600	A	BR 1998-14600	19981230
		WO 1998-CU8	19981230
AU 2003208135	A1 Div ex	AU 1999-19591	19981230
		AU 2003-208135	20030703
US 6723323	B1	WO 1998-CU8	19981230
		US 2000-582772	20001204

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9919591	A	Based on	WO 9935271
EP 1099759	A2	Based on	WO 9935271
JP 2002500047	W	Based on	WO 9935271
BR 9814600	A	Based on	WO 9935271
US 6723323	B1	Based on	WO 9935271

PRIORITY APPLN. INFO: CU 1997-142 19971230; AU 2003-208135 20030703

AN 1999-430398 [36] WPIDS

AB WO 9935271 A UPAB: 19990908

NOVELTY - Production, from a non-toxigenic strain of Vibrio **cholerae**, of innocuous derivatives suitable for immunization against cholera comprises inactivating the gene for hemagglutinin protease (HP), either by deletion, insertion or some other defined and irreversible genetic manipulation.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) vaccine strain of V. **cholerae** produced this way;

(2) method for producing biologically safe derivatives of innocuous vaccinating strains of V. **cholerae** by introducing a defined and irreversible mutation into the gene for thymidilate synthase (TS);

V. **cholerae** strains produced by method (2); and

(3) pure DNA (I) containing the sequence for the natural **thyA** gene of V. **cholerae**.

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Induction of specific immune response. Strain 638 (derived from the El Tor (Ogawa) strain 81 by insertional inactivation of the HP gene) was administered to 42 subjects. 34 became positive for specific bactericidal antibodies. Only four subjects developed diarrhea.

USE - The new strains are used to produce anticholera vaccines.

ADVANTAGE - The method produces V. **cholerae** strains that are genetically defined and stable, also suitable for oral administration. Eliminating the mucinase activity associated with HP means that the cells can not easily enter enterocytes (having a thick mucin layer), so have reduced reactogenic potential, but can still enter M cells to generate an immune response. Strains in which TS is also deleted are auxotrophic for thymidine so are unlikely to survive if they escape into the environment.

DESCRIPTION OF DRAWING(S) - Map of plasmid pGPH6 used to generate HP-deleted strains, showing the HP gene (hap) disrupted by the celA marker gene.

Dwg.4/5

L4 ANSWER 9 OF 11 MEDLINE on STN DUPLICATE 7

ACCESSION NUMBER: 96123352 MEDLINE

DOCUMENT NUMBER: PubMed ID: 8559036

TITLE: Thymine auxotrophy as an attenuating marker in Vibrio **cholerae**.

AUTHOR: Attridge S R

CORPORATE SOURCE: Department of Microbiology and Immunology, University of Adelaide, South Australia.

Searcher : Shears 571-272-2528

SOURCE: Microbial pathogenesis, (1995 Jul) 19 (1) 11-8.
Journal code: 8606191. ISSN: 0882-4010.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199602
ENTRY DATE: Entered STN: 19960312
Last Updated on STN: 19960312
Entered Medline: 19960223

AB Vibrio **cholerae** CVD102 is a thymine-dependent auxotroph of CVD101, a cholera toxin A-B+ candidate live oral cholera vaccine. Previous clinical experience with these strains suggested that, by restricting intestinal growth, thymine auxotrophy is attenuating for V. **cholerae**. Studies in the infant mouse cholera model cast doubt upon this conclusion however. Stable **thyA** mutants selected from each of three pathogenic strains showed unimpaired gut colonization in mixed-infection competition experiments. Similar results were obtained using **thyA** mutants selected from two atoxigenic strains, including CVD101. Further studies with CVD102 showed that the reduced colonization potential of this strain could not be compensated by the provision of a functional **thyA**+ gene in trans. CVD102 shows reduced synthesis of toxin-coregulated pili (TCP) during in vitro growth, suggesting the presence of a second, undefined mutation in this strain. Given the critical role of TCP in intestinal colonization, it seems probable that this previously unrecognized mutation is responsible for the poor in vivo performance of CVD102.

L4 ANSWER 10 OF 11 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 92077424 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1720753
TITLE: Construction of plasmid vectors with a non-antibiotic selection system based on the Escherichia coli **thyA**+ gene: application to cholera vaccine development.
AUTHOR: Morona R; Yeadon J; Considine A; Morona J K; Manning P A
CORPORATE SOURCE: Enterovax Limited, University of Adelaide, South Australia.
SOURCE: Gene, (1991 Oct 30) 107 (1) 139-44.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199201
ENTRY DATE: Entered STN: 19920202
Last Updated on STN: 19980206
Entered Medline: 19920113

AB The construction of live oral carriers based on attenuated Salmonella strains as vectors offers a new approach to vaccine development. We have constructed a set of plasmid vectors which have the **thyA** gene of Escherichia coli (encoding thymidylate synthetase) as the marker for selection and maintenance of plasmid clones. The **thyA** system offers an alternative

to antibiotic-resistance selection markers. It can be easily adapted to a particular host-vector combination since **thyA** chromosomal mutations can be readily introduced by trimethoprim selection. We also describe the construction of **thyA** -based plasmids with the Vibrio **cholerae** rfb genes (encoding O-antigen biosynthesis of the Inaba serotype). These have been found to be useful in the construction of candidate bivalent cholera-typhoid vaccines.

L4 ANSWER 11 OF 11 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: 1990-139807 [19] WPIDS
DOC. NO. CPI: C1990-061379
TITLE: New avirulent Salmonella containing DNA - for E. coli lipo polysaccharide core region, providing efficient expression of heterologous, especially cholera 10-antigen.
DERWENT CLASS: B04 D16
PATENT ASSIGNEE(S): (ENTE-N) ENTEROVAX LTD
COUNTRY COUNT: 2
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
AU 8941023	A	19900308	(199019)*		
US 5110588	A	19920505	(199221)		43

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
AU 8941023	A	AU 1988-941023	19880901
US 5110588	A	US 1989-401403	19890901

PRIORITY APPLN. INFO: AU 1986-7545 19860819; AU 1988-186 19880901; AU 1988-1273 19881102
AN 1990-139807 [19] WPIDS
AB AU 8941023 A UPAB: 19930928
The following bacteria are new (1) a virulent strain of Salmonella contg a DNA fragment encoding at least part of the core region of an E.coli strain; (2) the E.coli donor strains EX170, EX173 and EX260 (or their variants and mutants); and (3) the Salmonella donor strains S. typhimurium V490 and S.typhi V487.

More specifically a virulent Salmonella-E.coli composites include a **thyA** a virulent strain of S. typhi into which have been inserted (a) a DNA fragment contg genes, including the rfa locus, located at about 81 min on the E coli K12 genetic map, and including enzymes required for making the core region of the lipopalysaccharide (LP5), and (b) a DNA fragment able to express an 0-antigen and having a **thy A**+ non-antibiotic marker.

USE/ADVANTAGE - The composites are useful as m vaccines for protection against enteric diseases, esp cholera (the antigen being expressed is then Vibrio **cholerae** 0-somatic antigen, VcOAg). They provide higher levels of antigen prodn than unmodified

Salmonella strains and also express Salmonella O-somatic antigen. @ 0/15

ABEQ US 5110588 A UPAB: 19930928
Vaccine compsn. comprises an avirulent Salmonella-Escherichia coli hybrid strain obtd. by modifying an avirulent Salmonella strain by genetic engineering, such that the lipopolysaccharide core region of the hybrid is the E. coli lipopolysaccharide core region and the hybrid produces a Vibrio **cholerae** O-antigen; dispersed with the usual carriers and opt. additives.
USE - The prods. are vaccines against enteric bacterial infections.

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXCENTER, PASCAL, DISSABS, FEDRIP' ENTERED AT 14:27:47 ON 30 JUL 2004)

L5 292 S "CARLIN N"?/AU
L6 148 S "LEBENS M"?/AU -Author(s)
L7 7 S L5 AND L6
L8 3 S (L5 OR L6) AND L1
L9 7 S L7 OR L8
L10 2 DUP REM L9 (5 DUPLICATES REMOVED)

L10 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2002:589310 CAPLUS
DOCUMENT NUMBER: 137:305539
TITLE: The nptA gene of Vibrio cholerae encodes a functional sodium-dependent phosphate cotransporter homologous to the type II cotransporters of eukaryotes
AUTHOR(S): **Lebens, Michael**; Lundquist, Patrik; Soderlund, Lars; Todorovic, Mirjana; **Carlin, Nils I. A.**
CORPORATE SOURCE: Department of Medical Microbiology and Immunology, University of Goteborg, Goteborg, SE-431 46, Swed.
SOURCE: Journal of Bacteriology (2002), 184(16), 4466-4474
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The nptA gene of Vibrio cholerae has significant protein sequence homol. with type II sodium-dependent phosphate (Pi) cotransporters found in animals but not previously identified in prokaryotes. The phylogeny of known type II cotransporter sequences indicates that nptA may be either an ancestral gene or a gene acquired from a higher eukaryotic source. The gene was cloned into an expression vector under the control of an inducible promoter and expressed in Escherichia coli. The results demonstrate that nptA encodes a functional protein with activity similar to that of the animal enzyme, catalyzing high-affinity, sodium-dependent Pi uptake with comparable affinities for both sodium and phosphate ions. Furthermore, the activity of NptA is influenced by pH, again in a manner similar to that of the NaPi-2a subtype of the animal enzyme, although it lacks the corresponding REK motif thought to be responsible for this phenomenon. Pi uptake activity, a component of

which appeared to be sodium dependent, was increased in V. cholerae by phosphate starvation. However, it appears from the use of a reporter gene expressed from the nptA promoter that none of this activity is attributable to the induction of expression from nptA. It is thus proposed that the physiol. function of NptA protein may be the rapid uptake of Pi in preparation for rapid growth in nutrient-rich environments and that it may therefore play a role in establishing infection.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 1999:764211 CAPLUS
DOCUMENT NUMBER: 132:19629
TITLE: Production of **ThyA**- strains of Vibrio cholerae and their use for expression of heterologous proteins
INVENTOR(S): **Carlin, Nils; Lebens, Michael R.**
PATENT ASSIGNEE(S): SBL Vaccin AB, Swed.
SOURCE: PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9961634	A1	19991202	WO 1999-EP3509	19990521
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2329255	AA	19991202	CA 1999-2329255	19990521
AU 9944999	A1	19991213	AU 1999-44999	19990521
BR 9910703	A	20010130	BR 1999-10703	19990521
EP 1080211	A1	20010307	EP 1999-927745	19990521
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, LT, LV, FI				
EE 200000686	A	20020415	EE 2000-686	19990521
NO 2000005950	A	20010125	NO 2000-5950	20001124

PRIORITY APPLN. INFO.: SE 1998-1852 A 19980526
WO 1999-EP3509 W 19990521

AB A method of producing a **thyA**-deficient (**thyA**-) strain of Vibrio cholerae comprising site-directed mutagenesis in the V. cholerae chromosome at the locus of the **thyA** gene. Particularly, a .DELTA.**thyA** strain of Vibrio cholerae lacking the thymidylate synthetase functionality of the **thyA** is disclosed. Knowledge of the **thyA** and surrounding

sequences allows the use of suitable suicide vectors for site-directed mutagenesis and strategies such as (1) insertional inactivation, (b) a combination of insertional inactivation and gene deletion, and (c) removal of the entire gene. The **thyA**- strain may comprise one or several episomal autonomously replicating DNA elements, such as plasmids, having an optionally foreign, e.g. Escherichia coli, functional **thyA** gene that enables the strain to grow in the absence of thymine in the growth medium, and optionally having a structural gene encoding a homologous or heterologous protein. Further, proteins encoded by a structural **thyA** gene and the 5'-flanking region are described. Addnl., a vaccine comprising a Vibrio cholerae .DELTA.**thyA** strain of the invention or a **thyA**- strain of Vibrio cholerae produced by the method of the invention is disclosed. Thus, a useful application of the **thyA** gene is in maintenance of recombinant plasmids employed in the overprodn. of recombinant proteins in V. cholerae, and in the use of the sequence for insertion of foreign genes in a selectable and site-specific manner into the V. cholerae chromosome. This system is exemplified by insertion and expression of the heat-labile enterotoxin B-subunit of Escherichia coli and the glutathione S-transferase 26-kDa protein of Schistosoma japonica.

FILE 'HOME' ENTERED AT 14:29:20 ON 30 JUL 2004

30jul04 13:29:14 User219783 Session D2037.2

SYSTEM:OS - DIALOG OneSearch
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Set	Items	Description
Set	Items	Description
S1	308	THYA OR (THY OR THYMINE)(W)A
S2	18	S1 AND CHOLERAE
S3	15	RD (unique items)

>>>No matching display code(s) found in file(s): 65, 113

- key terms

3/3,AB/1 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2004 Inst for Sci Info. All rts. reserv.

17032326 Document Delivery Available: 000185551200007 References: 30
TITLE: Construction and evaluation of a safe, live, oral Vibrio **cholerae** vaccine candidate, IEM108
AUTHOR(S): Liang WL; Wang SX; Yu FG; Zhang LJ; Qi GM; Liu YQ; Gao SY; Kan B (REPRINT)
AUTHOR(S) E-MAIL: kanb@btamail.net.cn
CORPORATE SOURCE: Chinese Ctr Dis Control & Prevent, Minist Hlth, POB 5/Beijing 102206//Peoples R China/ (REPRINT); Chinese Ctr Dis Control & Prevent, Minist Hlth, /Beijing 102206//Peoples R China/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2003, V71, N10 (OCT), P5498-5504
GENUINE ARTICLE#: 725PH
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: IEM101, a Vibrio **cholerae** 01 El Tor Ogawa strain naturally deficient in CTXPhi, was previously selected as a live cholera vaccine candidate. To make a better and safer vaccine that can induce protective immunity against both the bacteria and cholera toxin (CT), a new vaccine candidate, IEM108, was constructed by introducing a ctxB gene and an El Tor-derived rstR gene into IEM101. The ctxB gene codes for the protective antigen CTB subunit, and the rstR gene mediates phage immunity. The stable expression of the two genes was managed by a chromosome-plasmid lethal balanced system based on the housekeeping gene **thyA**. Immunization studies indicate that IEM108 generates good immune responses against both the bacteria and CT. After a single-dose intraintestinal vaccination with 10(9) CFU of IEM108, both anti-CTB immunoglobulin G and vibriocidal

antibodies were detected in the immunized-rabbit sera. However, only vibriocidal antibodies are detected in rabbits immunized with IEM101. In addition, IEM108 but not IEM101 conferred full protection against the challenges of four wild-type toxigenic strains of V. **cholerae** 01 and 4 mug of CT protein in a rabbit model. By introducing the rstR gene, the frequency of conjugative transfer of a recombinant El Tor-derived RS2 suicidal plasmid to IEM108 was decreased 100-fold compared to that for IEM101. This indicated that the El Tor-derived rstR cloned in IEM108 was fully functional and could effectively inhibit the El Tor-derived CTXPhi from infecting IEM108. Our results demonstrate that IEM108 is an efficient and safe live oral cholera vaccine candidate that induces antibacterial and antitoxic immunity and CTXPhi phage immunity.

3/3,AB/2 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

15633479 Document Delivery Available: 000181058800035 References: 53
TITLE: Construction and characterisation of O139 cholera vaccine candidates
AUTHOR(S): Ledon T (REPRINT); Valle E; Valmaseda T; Cedre B; Campos J; Rodriguez BL; Marrero K; Garcia H; Garcia L; Fando R
AUTHOR(S) E-MAIL: talena@biocnic.cneuro.edu.cu
CORPORATE SOURCE: Ctr Nacl Invest Cient, Grp Genet, AP 6412/Havana//Cuba/ (REPRINT); Ctr Nacl Invest Cient, Grp Genet, /Havana//Cuba/; Inst Finlay Sueros & Vacunas, /Havana 6412//Cuba/
PUBLICATION TYPE: JOURNAL
PUBLICATION: VACCINE, 2003, V21, N11-12 (MAR 7), P1282-1291
GENUINE ARTICLE#: 646WK
PUBLISHER: ELSEVIER SCI LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND
ISSN: 0264-410X
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The hemagglutinin/protease (HA/P) seems to be an attractive locus for the insertion of heterologous tags in live cholera vaccine strains. A DeltaCTXPhi spontaneous mutant derived from a pathogenic strain of O139 Vibrio **cholerae** was sequentially manipulated to obtain hapA : : celA derivatives which were later improved in their environmental safety by means of a **thyA** mutation. All the strains here obtained showed similar phenotypes in traits known to be remarkable for live cholera vaccines irrespective of their motility phenotypes, although the hapA mutants had a 10-fold decrease in their colonisation capacity compared with their parental strains in the infant mouse cholera model. However, the subsequent **thyA** mutation did not affect their colonisation properties in the same model. These preliminary results pave the way for further clinical assays to confirm the possibilities of these vaccine prototypes as safe and effective tools for the prevention of O139 cholera. (C) 2002 Published by Elsevier Science Ltd.

3/3,AB/3 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

12110436 References: 34

TITLE: Construction and characterization of a nonproliferative El Tor cholera vaccine candidate derived from strain 638
AUTHOR(S): Valle E; Ledon T; Cedre B; Campos J; Valmaseda T; Rodriguez B; Garcia L; Marrero K; Benitez J; Rodriguez S; Fando R (REPRINT)
AUTHOR(S) E-MAIL: Fando@biocnic.cneuro.edu.cu
CORPORATE SOURCE: Ctr Nacl Invest Cient, Grp Genet, AP 6990/La Habana//Cuba/ (REPRINT); Ctr Nacl Invest Cient, Grp Genet, /La Habana//Cuba/; Inst Finlay Sueros & Vacunas, /Havana//Cuba/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2000, V68, N11 (NOV), P6411-6418
GENUINE ARTICLE#: 366LN
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: In recent clinical assays, our cholera vaccine candidate strain, Vibrio **cholerae** 638 El Tor Ogawa, was well tolerated and immunogenic in Cuban volunteers. In this work we describe the construction of 638T, a thymidine auxotrophic version of improved environmental biosafety, In so doing, the **thyA** gene from V. **cholerae** was cloned, sequenced, mutated in vitro, and used to replace the wild-type allele, Except for its dependence on thymidine for growth in minimal medium, 638T is essentially indistinguishable from 638 in the rate of growth and morphology in complete medium, The two strains showed equivalent phenotypes with regard to motility, expression of the celA marker, colonization capacity in the infant mouse cholera model, and immunogenicity in the adult rabbit cholera model. However, the ability of this new strain to survive environmental starvation was limited with respect to that of 638, Taken together, these results suggest that this live, attenuated, but nonproliferative strain is a new, promising cholera vaccine candidate.

3/3,AB/4 (Item 4 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

06821054 References: 15
TITLE: THYMINE AUXOTROPHY AS AN ATTENUATING MARKER IN VIBRIO **CHOLERAE**
AUTHOR(S): ATTRIDGE SR
CORPORATE SOURCE: UNIV ADELAIDE,DEPT MICROBIOL & IMMUNOL,MICROBIAL PATHOGENESIS UNIT,GPO BOX 498/ADELAIDE/SA 5005/AUSTRALIA/ (Reprint)
PUBLICATION: MICROBIAL PATHOGENESIS, 1995, V19, N1 (JUL), P11-18
GENUINE ARTICLE#: RZ992
ISSN: 0882-4010
LANGUAGE: ENGLISH DOCUMENT TYPE: ARTICLE

ABSTRACT: Vibrio **cholerae** CVD102 is a thymine-dependent auxotroph of CVD101, a cholera toxin A(-)B(+) candidate live oral cholera vaccine. Previous clinical experience with these strains suggested that, by restricting intestinal growth, thymine auxotrophy is attenuating for V. **cholerae**. Studies in the infant mouse cholera model cast doubt upon this conclusion however. Stable **thyA** mutants selected from each of three pathogenic strains showed unimpaired gut colonization in mixed-infection competition experiments. Similar results were obtained using **thyA** mutants selected from two atoxigenic strains, including

CVD101. Further studies with CVD102 showed that the reduced colonization potential of this strain could not be compensated by the provision of a functional **thyA**(+) gene in trans. CVD102 shows reduced synthesis of toxin-coregulated pill (TCP) during in vitro growth, suggesting the presence of a second, undefined mutation in this strain. Given the critical role of TCP in intestinal colonization, it seems probable that this previously unrecognized mutation is responsible for the poor in vivo performance of CVD102. (C) 1995 Academic Press Limited

3/3,AB/5 (Item 5 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)

03298752 References: 23
TITLE: CONSTRUCTION OF PLASMID VECTORS WITH A NON-ANTIBIOTIC SELECTION SYSTEM BASED ON THE ESCHERICHIA-COLI **THYA+** GENE - APPLICATION TO CHOLERA VACCINE DEVELOPMENT
AUTHOR(S): MORONA R; YEADON J; CONSIDINE A; MORONA JK; MANNING PA
CORPORATE SOURCE: UNIV ADELAIDE,DEPT MICROBIOL & IMMUNOL,GPO BOX 498/ADELAIDE/SA 5001/AUSTRALIA/ (Reprint); ENTEROVAX LTD/ADELAIDE/SA 5001/AUSTRALIA/
PUBLICATION: GENE, 1991, V107, N1 (OCT 30), P139-144
GENUINE ARTICLE#: GU008
LANGUAGE: ENGLISH DOCUMENT TYPE: NOTE

ABSTRACT: The construction of live oral carriers based on attenuated Salmonella strains as vectors offers a new approach to vaccine development. We have constructed a set of plasmid vectors which have the **thyA** gene of Escherichia coli (encoding thymidylate synthetase) as the marker for selection and maintenance of plasmid clones. The **thyA** system offers an alternative to antibiotic-resistance selection markers. It can be easily adapted to a particular host-vector combination since **thyA** chromosomal mutations can be readily introduced by trimethoprim selection. We also describe the construction of **thyA**-based plasmids with the Vibrio **cholerae** rfb genes (encoding O-antigen biosynthesis of the Inaba serotype). These have been found to be useful in the construction of candidate bivalent cholera-typhoid vaccines.

3/3,AB/6 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01758014
6 human secreted proteins
Menschliches sekretiertes Protein
Proteine humaine secretee
PATENT ASSIGNEE:
Human Genome Sciences, (2534450), 9410 Key West Avenue, Rockville, Maryland 20850, (US), (Applicant designated States: all)
INVENTOR:
Ruben, Steven, 18528 Heritage Hills Drive, Olney MD 20832, (US)
Komatsoulis, George A., 9518 Garwood Street, Silver Spring MD 20901, (US)
Ni, Jian, 5502 Manorfield Road, Rockville MD 20853, (US)
Soppet, Daniel R., 15050 Stillfield Place, Centreville MD 22020, (US)

LEGAL REPRESENTATIVE:
VOSSIUS & PARTNER (100314), Siebertstrasse 4, 81675 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 1435361 A2 040707 (Basic)
EP 1435361 A3 040714
APPLICATION (CC, No, Date): EP 2004005133 001108;
PRIORITY (CC, No, Date): US 164731 P 991112; US 215132 P 000630
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE; TR
RELATED PARENT NUMBER(S) - PN (AN):
EP 1235907 (EP 2000977046)
INTERNATIONAL PATENT CLASS: C07K-014/47

ABSTRACT EP 1435361 A3
The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.
ABSTRACT WORD COUNT: 64
NOTE:
Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200428	1213
SPEC A	(English)	200428	109797
Total word count - document A			111010
Total word count - document B			0
Total word count - documents A + B			111010

3/3,AB/7 (Item 2 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01507832
METHOD OF DETECTING NUCLEIC ACID RELATING TO DISEASE
VERFAHREN ZUR ERKENNUNG VON NUKLEINSAURE IN BEZUG AUF ERKRANKUNGEN
PROCEDE DE DETECTION D'ACIDE NUCLEIQUE RELATIF A UNE MALADIE
PATENT ASSIGNEE:
Kabushiki Kaisha Toshiba, (2077102), 1-1, Shibaura 1-chome, Minato-ku, Tokyo 105-8001, (JP), (Applicant designated States: all)
INVENTOR:
HASHIMOTO, Koji, 4-12-703, Sagamihara 4-chome, Sagamihara-shi, Kanagawa 229-0031, (JP)
HASHIMOTO, Michie, 17-17-106, Jingumae 3-chome, Shibuya-ku, Tokyo 150-0001, (JP)
MISHIRO, Shunji, 24-9-301, Honkomagome 1-chome, Bunkyo-ku, Tokyo 113-0021, (JP)
OOTA, Yasuhiko, 13-11, Arai 1-chome, Nakano-ku, Tokyo 165-0026, (JP)
LEGAL REPRESENTATIVE:
Andrews, Timothy Stephen et al (78321), Marks & Clerk, 57-60 Lincoln's Inn Fields, London WC2A 3LS, (GB)

PATENT (CC, No, Kind, Date): EP 1375672 A1 040102 (Basic)
WO 2002077281 021003
APPLICATION (CC, No, Date): EP 2002702736 020305; WO 2002JP2030 020305
PRIORITY (CC, No, Date): JP 200190053 010327; JP 2001284112 010918
DESIGNATED STATES: DE; ES; FR; GB; GR; IT; SE
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: C12Q-001/68; C12N-015/09; C12M-001/00; G01N-033/53; G01N-033/543; G01N-033/566; G01N-033/576; G01N-037/00

ABSTRACT EP 1375672 A1
The present invention provides methods for obtaining information regarding nucleic acid from an individual and nucleic acid associated with a disease of the individual, in particular when the disease is associated with a pathogenic microorganism present within the individual. The present invention also provide probe-immobilized substrates, such as probe-immobilized chips, for use in the methods. In particular, the present invention provides methods and probe-immobilized substrates for obtaining information regarding responsiveness to a treatment for a disease of an individual.
ABSTRACT WORD COUNT: 80
NOTE:
Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; Japanese
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200401	7143
SPEC A	(English)	200401	11956
Total word count - document A			19099
Total word count - document B			0
Total word count - documents A + B			19099

3/3,AB/8 (Item 3 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01114317
METHOD OF PRODUCING **THY-A**-DEFICIENT STRAINS OF VIBRIO **CHOLERAE**, SUCH STRAINS AND THEIR USE
VERFAHREN ZUR HERSTELLUNG **THY-A** DEFIZIENTE VIBRIO **CHOLERAE** STAMME, SOLCHE STAMME UND DEREN VERWENDUNG
METHODE DE PRODUCTION DE SOUCHES $i(**THY**) **A**?- DE $i(VIBRIO **CHOLERAE**), LESDITES SOUCHES ET LEUR UTILISATION
PATENT ASSIGNEE:
Active Biotech AB, (2744720), Box 724, 220 07 Lund, (SE), (Applicant designated States: all)
INVENTOR:
CARLIN, Nils, Stallknektsgrand 14, S-165 57 Hasselby, (SE)
LEBENS, Michael, R., Dr. Belfrages Vag 20, S-413 22 Goteborg, (SE)
LEGAL REPRESENTATIVE:
Woods, Geoffrey Corlett et al (48721), J.A. KEMP & CO. Gray's Inn 14 South Square, London WC1R 5JJ, (GB)
PATENT (CC, No, Kind, Date): EP 1080211 A1 010307 (Basic)
WO 9961634 991202
APPLICATION (CC, No, Date): EP 99927745 990521; WO 99EP3509 990521

PRIORITY (CC, No, Date): SE 981852 980526
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
EXTENDED DESIGNATED STATES: LT; LV
INTERNATIONAL PATENT CLASS: C12N-015/74; C12N-015/54; C12N-015/31; C12N-009/10; C07K-014/245; C07K-014/28; C07K-014/435; A61K-039/106; A61K-039/108; C12R-1:63
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English

3/3,AB/9 (Item 4 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01071957
VIBRIO **CHOLERAE** VACCINE CANDIDATES AND METHODS OF THEIR CONSTRUCTING
VIBRIO **CHOLERAE** IMPFSTOFFKANDIDATEN UND VERFAHREN ZU DEREN HERSTELLUNG
NOUVEAUX CANDIDATS VACCINS CONTRE LE VIBRIO **CHOLERAE** ET LEUR PROCEDE D'OBTENTION
PATENT ASSIGNEE:
CENTRO NACIONAL DE INVESTIGACIONES CIENTIFICAS, (1437833), Avenida 25, No. 15819,esq. a 158 Cubanacan, Playa, Ciudad de la Habana 12100, (CU), (Applicant designated States: all)
INVENTOR:
CAMPOS GOMEZ, Javier, Villuendas No.408 ent.Sindico y Caridad, Vil.Clara, Santa Clara 50100, (CU)
FANDO CALZADA, Rafael Alfredo, Ap.41, Cal.43 No 5838 ent.58B-58C, R. Ceiba,Playa, Ciudad de la Habana 11400, (CU)
RODRIGUEZ GONZALEZ, Boris Luis, Ap.6K ent.31-33, Cal.186 No. 3117, Playa, Ciudad de la Habana 11500, (CU)
LEDON PEREZ, Talena Yamile, Cal.Maloja No.270 ent.Lealtad y Campanario, Centro, Ciudad de la Habana 12400, (CU)
VALLE DIAZ, Edgar, Avenida Finlay No. 464 ent.1-3, Reparto Puerto Pr., Camaguey 70800, (CU)
SILVA CABRERA, Anisia Juana, Cal.208 No.1935 ent.19-21, Atabey, Playa, Ciudad de la Habana 12100, (CU)
BENITEZ ROBLES, Jorge Antonio, Cal.208 No.1935 ent.19-21, Atabey, Playa, Ciudad de la Habana 12100, (CU)
LEGAL REPRESENTATIVE:
Braun, Andre (40063), BRAUN & PARTNER Patent-, Marken-, Rechtsanwalte Reussstrasse 22, 4054 Basel, (CH)
PATENT (CC, No, Kind, Date): EP 1099759 A2 010516 (Basic)
WO 9935271 990715
APPLICATION (CC, No, Date): EP 98964347 981230; WO 98CU8 981230
PRIORITY (CC, No, Date): CU 14297 971230
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: C12N-015/31; C12N-001/21; A61K-039/106; C12N-009/10; C12N-001/21; C12R-1:63

ABSTRACT EP 1099759 A2
Vibrio **cholerae** vaccine strains which have a disrupted hap gene

and which are tagged with celA coding functions from Clostridium thermocellum are described. A contained, genetically defined **thyA** mutant of Vibrio **cholerae** and the general methodology of making along with the sequence of **thyA** gene are also described.
ABSTRACT WORD COUNT: 49
NOTE:
Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; Spanish
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	200120	491
SPEC A	(English)	200120	4671
Total word count - document A			5162
Total word count - document B			0
Total word count - documents A + B			5162

3/3,AB/10 (Item 5 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00269381
Hybrid bacterial strain.
Hybrider Bakterienstamm.
Souche hybride bacterienne.
PATENT ASSIGNEE:
ENTEROVAX RESEARCH PTY. LTD., (392591), University of Adelaide, North Terrace Adelaide South Australia, (AU), (applicant designated states: AT;BE;CH;DE;ES;FR;GB;GR;IT;LI;LU;NL;SE)
INVENTOR:
Morona,Renato, 10 Talbot Road, Waterloo Corner,South Australia, (AU)
LEGAL REPRESENTATIVE:
Harding, Richard Patrick et al , Arthur R. Davies & Co. 27 Imperial Square, Cheltenham GL50 1RQ, (GB)
PATENT (CC, No, Kind, Date): EP 257837 A1 880302 (Basic)
APPLICATION (CC, No, Date): EP 87306833 870731;
PRIORITY (CC, No, Date): AU 867545 860819
DESIGNATED STATES: AT; BE; CH; DE; ES; FR; GB; GR; IT; LI; LU; NL; SE
INTERNATIONAL PATENT CLASS: C12N-015/00; A61K-039/108; A61K-039/112; A61K-039/106;

ABSTRACT EP 257837 A1
An avirulent strain of Salmonella including a fragment of DNA containing genes encoding the synthesis of at least a portion of the core region of an E.coli strain.
The avirulent strain may form the basis of a live oral vaccine against cholera.
ABSTRACT WORD COUNT: 46

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	744
SPEC A	(English)	EPABF1	3770
Total word count - document A			4514

Total word count - document B 0
Total word count - documents A + B 4514

3/3,AB/11 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0324345 DBR Accession No.: 2003-25486
Construction and evaluation of a safe, live, oral Vibrio **cholerae** vaccine candidate, IEM108 - vector plasmid expression in host cell for use in bacterium vaccine
AUTHOR: LIANG WL; WANG SX; YU FG; ZHANG LJ; QI GM; LIU YQ; GAO SY; KAN B
CORPORATE AFFILIATE: Chinese Ctr Dis Control and Prevent
CORPORATE SOURCE: Kan B, Chinese Ctr Dis Control and Prevent, Natl Inst Communicable Dis Control and Prevent, Prior Lab Med Mol Bacteriol, Minist Hlth, POB 5, Beijing 102206, Peoples R China
JOURNAL: INFECTION AND IMMUNITY (71, 10, 5498-5504) 2003
ISSN: 0019-9567
LANGUAGE: English
ABSTRACT: AUTHOR ABSTRACT - IEM101, a Vibrio **cholerae** 01 El Tor Ogawa strain naturally deficient in CTXPhi, was previously selected as a live cholera vaccine candidate. To make a better and safer vaccine that can induce protective immunity against both the bacteria and cholera toxin (CT), a new vaccine candidate, IEM108, was constructed by introducing a ctxB gene and an El Tor-derived rstR gene into IEM101. The ctxB gene codes for the protective antigen CTB subunit, and the rstR gene mediates phage immunity. The stable expression of the two genes was managed by a chromosome-plasmid lethal balanced system based on the housekeeping gene **thyA**. Immunization studies indicate that IEM108 generates good immune responses against both the bacteria and CT. After a single-dose intraintestinal vaccination with 10(9) CFU of IEM108, both anti-CTB immunoglobulin G and vibriocidal antibodies were detected in the immunized-rabbit sera. However, only vibriocidal antibodies are detected in rabbits immunized with IEM101. In addition, IEM108 but not IEM101 conferred full protection against the challenges of four wild-type toxigenic strains of V. **cholerae** 01 and 4 mug of CT protein in a rabbit model. By introducing the rstR gene, the frequency of conjugative transfer of a recombinant El Tor-derived RS2 suicidal plasmid to IEM108 was decreased 100-fold compared to that for IEM101. This indicated that the El Tor-derived rstR cloned in IEM108 was fully functional and could effectively inhibit the El Tor-derived CTXPhi from infecting IEM108. Our results demonstrate that IEM108 is an efficient and safe live oral cholera vaccine candidate that induces antibacterial and antitoxic immunity and CTXPhi phage immunity. (7 pages)

3/3,AB/12 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0248511 DBR Accession Number: 2000-03001 PATENT
New Vibrio **cholerae** strain defective in the **thyA** gene, for use in vaccines and for recombinant protein production - mutant Vibrio **cholerae** used to produce large amounts of recombinant protein or

as a cholera vaccine
AUTHOR: Carlin N; Lebens M R
CORPORATE SOURCE: Stockholm, Sweden.
PATENT ASSIGNEE: SBL-Vaccin 1999
PATENT NUMBER: WO 9961634 PATENT DATE: 19991202 WPI ACCESSION NO.: 2000-062719 (2005)
PRIORITY APPLIC. NO.: SE 981852 APPLIC. DATE: 19980526
NATIONAL APPLIC. NO.: WO 99EP3509 APPLIC. DATE: 19990521
LANGUAGE: English
ABSTRACT: A Vibrio **cholerae** strain that lacks **thyA** gene function, is claimed. Also claimed is a means of producing that V. **cholerae** strain by site-directed mutagenesis, the **thyA** gene (A) with a given 2,909 bp DNA sequence, the given 838 bp DNA sequence that forms the 5' flanking region of (A), the given 1,222 bp DNA sequence that forms the 3' flanking region of (A), a given 283 amino acid protein sequence encoded by (A), a given 271 amino acid protein sequence, and a vaccine containing the modified V. **cholerae** strain. The **thyA**-negative V. **cholerae** can be used for the overproduction of recombinant proteins, and in vaccines against cholera. The V. **cholerae** strain produces high levels of recombinant proteins, which it secretes into the culture medium. The V. **cholerae** strain preferably has the entire **thyA** gene deleted, but includes an episomal, autonomously replicating DNA element encoding **thyA** , particularly on a plasmid, and so can grow in thymidine-deficient medium. The **thyA** mutant was preferably generated by removing the **thyA** gene using suicide vector plasmid pMT-SUICIDE-1. (42pp)

3/3,AB/13 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0117936 DBR Accession Number: 91-05578
Construction and efficacy of a live oral bacterial cholera-typhoid vaccine - Vibrio **cholerae** O-antigen lipopolysaccharide gene cloning and expression in attenuated Salmonella typhi; multivalent recombinant vaccine construction (conference paper)
AUTHOR: Attridge S; Forrest B; Hackett J; la Brooy J; Levine M M; Morona R
CORPORATE AFFILIATE: Enterovax
CORPORATE SOURCE: Enterovax LImited, Adelaide, Australia.
JOURNAL: Aust.Biotechnol.Conf. (8 Meet., 134-39) 1989
CODEN: 9999Z
LANGUAGE: English
ABSTRACT: A live oral bacterial cholera-typhoid vaccine was constructed. Salmonella typhi Ty21a, a safe and moderately immunogenic attenuated derivative of S. typhi Ty2, was used as a host. S. typhi EX645, containing recombinant plasmid pEVX22, and having a spontaneous rifampin-resistance mutation, a non-reverting **thyA** mutation, and up to 200 kb Escherichia coli K-12 DNA, including rfa genes, to replace S. typhi DNA, was constructed. Plasmid pEVX22 contained 20 kb of DNA from Vibrio **cholerae** 569B, in plasmid pSC101, and encoded production of V. **cholerae**-like O-antigen lipopolysaccharide by the recombinant. The recombinant strain was safe when administered orally to humans, and induced immune responses against

lipopolysaccharide of both S. typhi and V. **cholerae**. Of 8 subjects immunized, 2 were fully protected from cholera, and the others were partially protected. Modified strains with increased immunogenicity were constructed (e.g. S. typhi EX879 and S. typhi EX880). (6 ref)

3/3,AB/14 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0107852 DBR Accession Number: 90-10543 PATENT
New avirulent Salmonella containing DNA - for Escherichia coli lipopolysaccharide core region, providing efficient expression of heterologous protein, especially cholera 0-antigen; application as vaccine
PATENT ASSIGNEE: Enterovax 1990
PATENT NUMBER: AU 8941023 PATENT DATE: 900308 WPI ACCESSION NO.: 90-139807 (9019)
PRIORITY APPLIC. NO.: AU 89186 APPLIC. DATE: 890901
NATIONAL APPLIC. NO.: AU 88941023 APPLIC. DATE: 880901
LANGUAGE: English
ABSTRACT: The following bacteria are new: (1) a virulent strain of Salmonella containing a DNA fragment encoding at least part of the core region of an Escherichia coli strain; (2) the E. coli donor strains EX170, EX173 and EX260, or their variants and mutants; and (3) the Salmonella donor strains Salmonella typhimurium V490 and Salmonella typhi V487. A Salmonella-E.coli composition includes a **thyA** avirulent strain of S. typhi into which (a) a DNA fragment containing genes, including the rfa locus located at 81 min on the E. coli K12 genetic map, and including enzymes required for making the core region of the lipopolysaccharide, and (b) a DNA fragment able to express an 0-antigen and having a **thyA**+ non-antibiotic selectable marker, are inserted. The modified Salmonella strains are useful as vaccines for protection against enteric diseases, especially cholera (where the antigen being expressed is Vibrio **cholerae** 0-somatic antigen. They provide higher levels of antigen production than unmodified Salmonella strains and also express Salmonella 0-somatic antigen. (98pp)

3/3,AB/15 (Item 5 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0072496 DBR Accession Number: 88-03345 PATENT
Non-antibiotic marker system - novel plasmid expressing recombinant Vibrio **cholerae** or Salmonella genes for cholera, etc. vaccine production
PATENT ASSIGNEE: Enterovax-Res. 1988
PATENT NUMBER: EP 251579 PATENT DATE: 880107 WPI ACCESSION NO.: 88-001444 (8801)
PRIORITY APPLIC. NO.: AU 866553 APPLIC. DATE: 860624
NATIONAL APPLIC. NO.: EP 87305404 APPLIC. DATE: 870618
LANGUAGE: English
ABSTRACT: A novel plasmid that does not encode antibiotic-resistance includes a suitable plasmid cloning vector and a first cloned fragment

of DNA containing an attached, non-reverting **thyA** + gene. Preferably the first cloned DNA fragment is a HindIII fragment from plasmid pBTAH containing the **thyA**+ gene. Preferably at least 1 antibiotic-resistance coding region on the cloning vector is inactivated by the insertion or otherwise inactivated, especially using an enzyme. The inactivated antibiotic-resistance region is especially ampicillin- and/or tetracycline-resistance. The cloning vector is selected from pSC101, pUC18, pUC19, pBR322 and pBTAH, especially pBR322. The claimed recombinant plasmid also contains a gene from a pathogen of human or animal importance, especially a DNA fragment from pOmpV210 encoding the Vibrio **cholerae** outer membrane protein and/or the O-antigen. The recombinant plasmid is pEVX1, pEVX2, etc. Also claimed is a plasmid for expression of Salmonella spp. genes. The plasmids are useful for the production of vaccines against cholera, typhoid, etc. (14pp)

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Set     Items   Description
S4        217   AU=(CARLIN, N? OR CARLIN N?)
S5         55   AU=(LEBENS, M? OR LEBENS M?)
S6          5   S4 AND S5
S7          2   (S4 OR S5) AND S1
S8          3   (S6 OR S7) NOT S2
S9          1   RD (unique items)
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The nptA Gene of Vibrio cholerae Encodes a Functional Sodium-Dependent Phosphate Cotransporter Homologous to the Type II Cotransporters of Eukaryotes

Lebens, M.; Lundquist, P.; Soderlund, L.; Todorovic, M.; **Carlin**, **N. I. A.**

CONFERENCE: Microbial genomes; Microbial genomics-challenges and opportunities -International conference; 9th

JOURNAL OF BACTERIOLOGY, 2002; VOL 184; NO 16 P: 4466-4474

ASM, 2002

ISSN: 0021-9193

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CONFERENCE LOCATION: Gatlinburg, TN 2001; Oct (200110) (200110)

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